IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Dominique BERNARD et al.

Application No.: 09/884,953

Filed: June 21, 2001

For: ISOLATED CATHEPSIN L TYPE

CYSTEINE PROTEASES AND

REDUCING INTERCORNEOCYTE

COHESION/PROMOTING
DESQUAMATION THEREWITH

Creation No.: 1651

Examiner: Jon P. Weber

Confirmation No.: 3212

Confirmation No.: 3212

## AMENDMENT/REPLY TRANSMITTAL LETTER

Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

Sir:

Encl	osed is a Supplemental Response for the above-identified patent application.
[]	A Petition for Extension of Time is also enclosed.
[]	A Terminal Disclaimer and the [ ] \$55.00 (2814) [ ] \$110.00 (1814) fee due under 37 C.F.R. § 1.20(d) are also enclosed.
[X]	Also enclosed is/are Annexes 1-8, Information Disclosure Statement Transmittal Letter Information Disclosure Statement, and PTO 1449 with copies of references.
[]	Small entity status is hereby claimed.
[]	Applicant(s) requests continued examination under 37 C.F.R. § 1.114 and enclose the [ ] \$375.00 (2801) [ ] \$750.00 (1801) fee due under 37 C.F.R. § 1.17(e).
	[ ] Applicant(s) requests that any previously unentered after final amendments <u>not</u> be entered. Continued examination is requested based on the enclosed documents identified above.
	[ ] Applicant(s) previously submitted, on, for which continued examination is requested.

Amendment/Reply Transmittal Letter Application No. <u>09/884,953</u> Attorney's Docket No. <u>016800-448</u> Page 2

[	]	Applicant(s) requests suspension of action by the Office until at least, which
		does not exceed three months from the filing of this RCE, in accordance with
		37 C.F.R. § 1.103(c). The required fee under 37 C.F.R. § 1.17(i) is enclosed.

- [ ] A Request for Entry and Consideration of Submission under 37 C.F.R. § 1.129(a) (1809/2809) is also enclosed.
- [X] No additional claim fee is required.
- [ ] An additional claim fee is required, and is calculated as shown below:

NO. OF CLAIMS EXTRA CLAIMS PAID FOR									
Total Claims MINUS = $\times$ \$18.00 (1202) =									
Independent Claims $MINUS = \times \$84.00 (1201) =$									
If Amendment adds mu	ltiple depend	lent claims, add \$28	30.00 (1203)						
Total Claim Amendmer	nt Fee								
If small entity status is	claimed, sub	tract 50% of Total	Claim Amend	ment Fee					

[	]	A total fee	in the amount of \$	is enclosed.
[	]	Charge \$_	to Deposit Account N	lo. 02-4800.

The Director is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17, 1.20(d) and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in duplicate.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: <u>July 9, 2003</u>

Jennifer A. Topmiller, Ph.

Registration No. 50,435

P.O. Box 1404 Alexandria, Virginia 22313-1404 (703) 836-6620 JUL 0 9 2003

# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)
Dominique BERNARD et al.	) Group Art Unit: 1651
Application No.: 09/884,953	) Examiner: Jon P. Weber
Filed: June 21, 2001	) Confirmation No.: 3212
For: ISOLATED CATHEPSIN L TYPE CYSTEINE PROTEASES AND REDUCING INTERCORNEOCYTE COHESION/PROMOTING DESQUAMATION THEREWITH	) ) ) )

## SUPPLEMENTAL RESPONSE

Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

Sir:

Further to the Amendment/Response filed June 26, 2003, submitted herewith are Annexes 1-8, inadvertently omitted from the Amendment/Response filed June 26, 2003.

If there are any questions concerning this Supplemental Response or the application in general, the Examiner is respectfully requested to telephone Applicants' undersigned representative so that prosecution may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: \_July 9, 2003

lennifer A. Vopmiller, Ph.D.

Registration No. 50,435

P.O. Box 1404 Alexandria, Virginia 22313-1404 (703) 836-6620

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ANNEX 1

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# Compute pI/Mw

Annex 2 -

# CATL HUMAN (P07711)

DE CATHEPSIN L PRECURSOR (EC 3.4.22.15) (MAJOR EXCRETED PROTEIN) (MEP).

OS Homo sapiens (Human).

The computation has been carried out on the complete sequence.

Molecular weight: 37564.13

Theoretical pI: 5.32

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Page 1 del



# Compute pI/Mw

Ames 3

# CATL HUMAN (P07711)

DE CATHEPSIN L PRECURSOR (EC 3.4.22.15) (MAJOR EXCRETED PROTEIN) (MEP). OS Homo sapiens (Human).

The computation has been carried out on a user selected segment from position 114 to position 333 in this sequence of 333 residues.

Considered sequence fragment:

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	61	71	81	91	101	111	
	1	1	1	ŀ	1	1	
61						APR5VDW	120
121	REKGYVTPVK	NOGOCGSCWA	FSATGALEGO.	MFRKTGRLIS	1.SEQNLVDCS	GPQGNEGCNG	180
181	GLMDYAFQYV	ODNGGLDSEE	SYPYEATEES	CKYNPKYSVA	NDTGFVDIPK	QEKALMKAVA	240
241	TVGPISVAID	AGHESFLFYK	EGIYFEPDCS	SEDMDHGVLV	VGYGFESTES	DNNKYWLVKN	300
	SWGEEWGMGG						

Molecular weight: 24169.75

Theoretical pl: 4.68

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Quantity

1 ml

Polyclonal antibody to

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NICTRIBUTEUR

- valbiotech

57, BOULEYARD DE LA VILLETTE 75010 PARIS TEL : 33 (0)1 40 03 59 14 FAX : 33 (0)1 44 52 92 69

Host:

Immunogen:

Highly pure Cathepsin L (EC 3.4.22.15) from human kidney

Presentation:

Liquid, Ig fraction (by selective precipitation) in PBS, pH 7.2 without preservatives

ig concentration:

Assay system:

<u>2-6</u> mg/ml

ELISA/(not tested in IHC)

Titra

To be established in end user assay system using 1/2,500 to 1/10,000 as a guide in a simple ELISA

Cross reactivity:

Minimal cross reactivity with related serum, proteins. May cross react with Cathepsin L from other species.

Storage:

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Associated products:

Other conjugated antibodies to other Cathepsins, Cathepsin antigens.

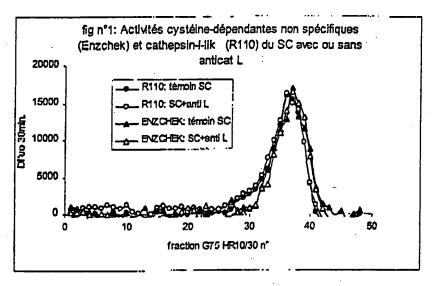
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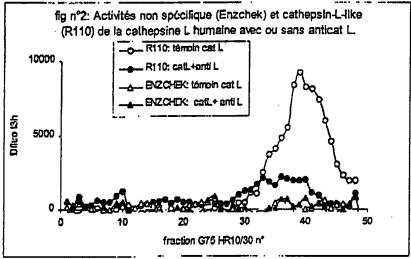
For In-Vitro Research and Manufacturing Use Only





Annex 5

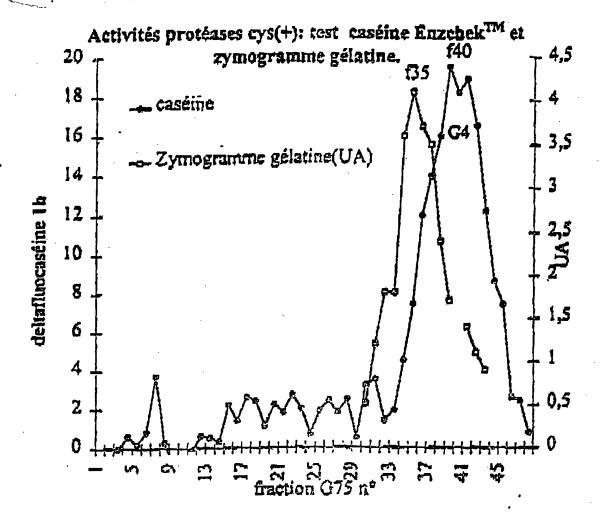




### Conclusions:

Ces résultats prouvent que la protéase cystéine-dépendante du SC humain détectée à la fois par le test ENZCHEK et par le substrat peptidique Z(Phe-Arg)<sub>2</sub>R110 est différente de la cathepsine L humaine car:

- 1) Elle n'est pas du tout immunoprécipitée par l'anticorps (sig n°1) alors que l'activité cathepsine L disparaît presque totalement des surnageants dans les mêmes conditions expérimentales (fig. n°2);
- 2) Elle a un poids moléculaire apparent plus grand que la cathepsine L comme le prouve son élution plus rapide (fig n°1/fig n°2);
- 3) Elle a une spécificité de substrat sans doute très différente de la cathepsine L car elle libère une fluorescence équivalente que ce soit à partir de la caséine du test Enzehek ou à partir du peptide marqué à la Rhodamine 110 (fig. n°)) alors que l'activité cathepsine L n'est détectable dans nos conditions expérimentales que sur le substrat peptidique (fig n°2).



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Biochem. J. (1988) 253, 703-306 (Printed in Great Britain)

# Isolation and sequence of a cDNA for human pro-(cathepsin L)

Susannah GAL\* and Michael M. GOTTESMANT Laborat ry of Molecular Biology, National Cancer Institute. National Institutes of Health, Building 37, Room 2518, Bethesda, MD 20892, U.S.A.

The major excreted protein (MEP) of malignantly transformed mouse fibroblasts is the precursor to an acid proteinase with enzymic specificity similar to that of human cathopsin L. By cross-hybridization with a mouse MEP sequence, cDNA clones of the human form of MEP in an SY40 expression vector were isolated. A 1.6 kb cDNA showed 70% deduced amino acid sequence identity with mouse MEP. The deduced amino acid sequence of the cloned human MEP was the same, except for two amino acids, as the N-terminal sequence of mature human cathepsin L, thereby establishing that human MEP is human pro-(enthepsin L). Use of this human pro-(cathepsin L) cDNA clone allowed the detection of a 1.6-1.8 kb pro-(cathepsin L) mRNA in human cells which was not detected with a mouse pro-(cathepsin L) probe.

#### INTRODUCTION

Monse fibroblasts which are malignantly transformed or stimulated by growth factors or tumour promoters synthesize and secrete increased amounts of a 39 kDa glycoprotein with acid-proteinase activity (Gottesman, 1978; Gottesman & Sobel, 1980; Doherty et al., 1985; Rabin et al., 1986; Frick et al., 1985; Nilson-Hamilton et al., 1981; Gal & Gottesman, 1986a). This protein, termed 'MEP' (major excited protein), is the precursor to two lower-M, lysosomal proteins of 29 kDa and 21 kDa (Gal et al., 1985), and contains the lycosomal recognition marker mannose 6-phosphate (Sahagian & Gottesman, 1982). Mouse MEP and human cathepsin L (Mason et al., 1985) share amino acid bond cleavage specificities, catalytic constants and inhibitor susceptibilities (Gal & Gottesman, 1986b; Mason et al., 1987). Comparison of the deduced amino acid sequences of mouse MEP, or a related protein called 'mouse systeins , with a partial amino acid sequence for human cathepsin L, indicates strong similarity between these two sequences, but because of the species differences, does not prove identity (Troen et al., 1987; Portnoy et al., 1986; Denhardt et al., 1986; Mason et al., 1986).

In the present paper, we report the isolation and characterization of a full-length human MEP cDNA clone, isolated by cross-hybridization with a mouse MEP cDNA clone. The deduced amino acid sequence from this clone was identical, except for two amino acids, with that of the human cathepsin L sequence, proving that MEP is cathepsin L. This human cathepsin L cDNA can be used as a probe to determine the expression of eathersin L mRNA in human cell lines and rissues.

# MATERIALS AND METHODS

Isolation of a human cDNA clone for cathepsin L

The Okayama-Berg (1983) cDNA expression library termed 'GM637', made from mRNA isolated from SV40-virus-transformed human fibroblests, was a gift

from Dr. Hirota Okayama (National Institute of Mental Health, National Institutes of Health, Bethesda, MD, U.S.A.). In all, 100000 clones were screened on 20 filters by the technique of Troen (1987) for colony hybridization, using the 800-bp restriction-endonuclease-EcoRI fragment of mouse MEP (Troen et al., 1987) labelled by nick translation (Loistrand Laboratories, Gaithersburg, MD, U.S.A.). The filters were washed under moderate stringency conditions: twice with 2×SSC (1×SSC = 0.15 M-NaCl/0.015 M-sodium citrate)/1% SDS and twice with 0.8 x SSC/1% SDS at 60 °C, and three positive clones were obtained. The clone designated pHu-16' contained the largest insert (1.6 kb) and was used for all subsequent studies.

# Sequencing and blotting

Sequencing was done using the Sanger et al. 1977) dideoxy method with the Promega sequencing kit (Promega Biotec, Madison, WI, U.S.A.) and deoxyadenoxine [a-185]thiotriphosphate at a specific radioactivity of 500 Ci/mmol (NEN, Boston, MA, U.S.A.). pHu-16 was sequenced by using a combination of two techniques: (1) subcloning regions of pHu-16 DNA into Promega vectors pGEM-3 and pGEM-4 with Sp6 and T7 primers (Promega), and (2) direct sequencing using 50 ng of synthetic oligonucleotides (Applied Biosystems) as primers and the entire pHu-16 cDNA as template.

#### Detection of human procethepsin L RNA

RNA was isolated from tissue-culture calls as previously described (Chirgwin et al., 1979; Maniatis et al., 1982), and Northern blots were as described by Shen et al. (1986). Northern blots were probed with nicktranslated EcoRI fragments from pHn-16 cDNA or pMMEP-14 cDNA (Troon et al., 1987, 1988).

### RESULTS AND DISCUSSION

Isolation and sequence of a human cathepsin L cDNA

We selected the human MEP clone by screening a cDNA library made from SV40-virus-transformed

Abbreviations used: MEP, major excreted protein Present address: Friedrich-Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland.

To whom correspondence and reprint (equals should be addressed.

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Fig. 1. (a) Sequencing strategy for pHu-16 and (b) the entire sequence of pHu-16 with the longest open reading frame translated underseath beginning at nucleotide 305 and terminating at nucleotide 1304

(a) Bold lines are sequences determined using subcloned fragments in pQEM3 or pQEM4 primed with Sp6 and T7 oligonnelsocides. The dotted lines indicate sequences derived from the entire pHv-16 plasmid using synthetic oligonuclastides (\*) as primers. The orientation of the clone is shown along with some of the restriction uties; V = PpuII, T = TaqI, E = EcoRI, R = RraI, S = SphI. (b) The poly(A) addition signal is underlined.

human fibroblasts at low stringency using an 800-bp EcoRI fragment from a mouse MEP cDNA clone (see the Materials and methods section). One clone, termed 'pHu-16', contained a 1600-bp cDNA insert. The restriction map, sequencing strategy, complete nucleotide sequence and deduced amino acid sequence for pHu-16 are shown in Fig. 1.

The sequence of the human MEP cDNA revealed a single long open reading frame coding for a protein of M, 37522. The deduced amino acid sequence was compared with the mouse MBP sequence (Fig. 2) and

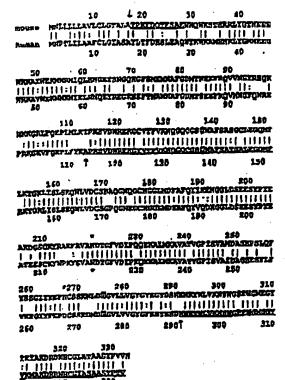


Fig. 2. Homology of mouse and human MEP sequences

320

Complete amino acid sequences deduced for pHu-16 (human MEP) and pMMEP-14 [mouse MEP (Troen et al., 1987)] are shown. The underlined sequences of human MEP correspond to protein sequences of human cathepsin L obtained by Muson et al. (1986). The underlined sequence of mouse MEP corresponds to the N-terminal sequence of secreted MEP purified from mouse tissue-culture medium (Troen et al., 1987). Arrows indicate where processing occurs, and asterisks indicate potential N-glycosylation sites. The proposed active-site amino celds haved on papein, Cys-138 and Hs-276, are shown by C and El respectively. Homology is indicated as exact matcher (I) and conservative substitutions (2).

was found to be 70% identical over the entire sequence, with some regions having 90% identity (e.g. between amino acids 115 and 168 and between amino acids 217 and 249). Over 98% sequence identity was found between the N-terminal amino acid sequence of mature human cathepsin L (Mason et al., 1986) and human MEP (shown as the underlined sequence in Fig. 2). The two amino acids which are different from our predicted sequence and that published by Mason et al. (Pro for Glu at position 148 and Tyr for Arg at position 153) may be due to problems with the amino acid analysis or to polymorphic differences in the proteins isolated from two different human sources. These results show that MEP is pro-(cathepsin L). Similar conclusions have been reached by Joseph et al. (1987), who reported the partial sequence of the human analogue of a ras-oncogene-induced protein which appears to be identical with MEP/cathepsin L.

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Fig. 3. Northern blots of RNA from tissue-culture cells and human tissues

(e) and (b), Northern blots of 10 µg of total RNA from tissue-culture och using two different probes. The sources of RNA were human KB ochs (a), multidrug-resistant KB cells (b), mouse NIH 373 cells (c) and multidrug-resistant KB cells (c) and multidrug-resistant KB cells (c) and multidrug-resistant NIH 173 cells (d) as previously described (Shen et al., 1986). In (a) hybridization was with the human phu-16 EcoRI fragment and is (b) hybridization was with the ScoRI fragment from the mouse MEP clone pMMEP-14 . (Troca et al., 1987).

As for mouse MEP and mouse cysteine proteinnee Troen et al., 1987; Portnoy et al., 1986; Denhardt et al., 1986), significant homologies were also observed when the human MEP sequence was compared with those of other cysteine proteinases, including cathepsin B, cathepsin H, actinidin and papain. Mouse and human forms of MEP show nearly identical sequences around amino acids Cys-138 and His-276 (Fig. 2), which are putative active sites on the basis of similarities to papain (Kamphuis et al., 1985). Comparison of our sequence with those of several other buman lysosomal proteins shows no obvious conscreed signals for the lysosomalprotein-specific transfer of UDP-N-acetylglucosamine to the high-mannose chain to yield the lysosomal mannose 6-phosphate marker.

The N-terminal sequence of secreted mouse MEF indicates where pre-MRP is processed to yield MEP (Troon et al., 1987). The deduced amino acid sequence of human prepro-(cathepsin L) also has the canonical Ala-Xaa-Ala sequence which precedes the leader-sequence cleavage site (Perlman & Halvorson, 1983) (Fig. 2). The positions of the N-terminal peptides of mature human cathepsin L within the deduced amino acid sequence of human MEP indicate the sites at which this precursor to human cathepsin L is processed to the lower M, mature forms.

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Because the full-length pro-(cathepsin L) cDNA which we isolated is cloned downstream from the SV40 promoter (Okayama & Berg, 1983), it is possible to express this cDNA in transfected cells. Recent experiments indicate that the human pro-(cathepsin L) cDNA is expressed in mouse NIH 3T3 cells as a 42 kDa protein that is secreted when it is overproduced (Kane et al.,

# Use of the human procathepsin L eDNA to detect a procethepsin L mRNA in human cells

ig, 3 shows the results of Northern blots with human KB (HeLz) cell and NIH 3T3 mouse-cell RNA probed with the EcoR1 800-bp fragment from the mouse and human MEP cDNA ciones. The human probe recognized a 1.6-1.8-kb pro-(cathepsin L) message in human KB cells which migrated just below the 18 S RNA marker (Fig. 3a) and appeared to be the same size as MEP mRNA from mouse cells (Fig. 3b). At high stringency, the mouse probe recognized a 1.6-1.8-kb RNA in mouse cells but no message in human cells (Fig. 3b). These data indicate that the human pro-(cathepsin L) cDNA described here (pHu-16) can be used to detect a human pro-(cathepsin L) mRNA, and that there is only one major pro-(cathepsin L) mRNA in cultured human KB cells. Preliminary results indicate that all normal human tissues tested express this pro-(cathepsin L) mRNA (results not shown). The availability of a full-length cDNA probe for human pro-(cathepsin L) will make it possible to assess relative levels of pro-(cathepsin L) mRNA in different tissues and in tumours.

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Annex 3(1)

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Your Ref: Our Ref: OA97128/JP/BN:CG JP1060LOR

Re

Japanese Patent Application No. Hei 10-244055
"Polypoptide isolated from the epidermis and its use"

Dear Mr. Galup:

Thank you for the front page of JP-A-H06-192124 (Toray). The following is an English translation of the paragraphs [0012] and [0013].

[0012]

Human cathensin L is already known as one type of thiol protease (Mason et al., Biochemical Journal, 240, 373-377, 1986), and its ability of clustin degradation (Muson et al., Biochemical Journal, 233, 925-927, 1986) and an action of inactivating ~ 1-protease inhibitor (Johnson et al., Journal of Biological Chemistry, 261, 14748-14751, 1986) have been reported.
[8013]

The prepro form of human cathepsin L consists of 333 amino acid residues (molecular weight of 38000), and the precursor (pro form) is the one in which N-terminal 1-17 residues are deleted. Namely, it comprises 18-333 residues (316 amino acid residues with molecular weight of 36000), and N-terminal amino acid sequence is as shown in Seq ID No: 1 of the sequencing list (Joseph et al., J. Clinic. Investigation, 31, 1621-1629, 1988). Among these, 18-113 residues are called activation peptide, 114-288 residues are a cathepsin H heavy chain, and 292-333 residues are a cathepsin L chain.

According to the paragraph [0015], the cathepsin L of the present invention includes precursor (18-333 residues), mature form (114-333 residues), H-chain (114-288 residues), L-chain (292-333 residues), and the precursor is preferable. Thus, the authors preferably use the procathepsin L. With respect to Mason et al., Biochemical Journal, 233, 925-927, 1986 or Johnson et al., Journal of Biological Chemistry, 261, 14748-14751, 1986, it appears from the context of the above two paragraphs that they describe human outhopsin L or its prepro form or proferm or the like which are mentioned in the paragraph [0013]. Therefore, we presume that the human cathepsin L described in these documents are the same as those used by the authors.

We hope we have answered your question sufficiently, but should you have any questions, please do not healtate to contact us.

Very truly yours,

Yozninori Kobayashi